Reassessing *Escherichia coli* as a cell factory for biofuel production

Chonglong Wang[1], Brian F Pfleger[2,3] and Seon-Won Kim[4]

CrossMark

Via metabolic engineering, industrial microorganisms have the potential to convert renewable substrates into a wide range of biofuels that can address energy security and environmental challenges associated with current fossil fuels. The user-friendly bacterium, *Escherichia coli*, remains one of the most frequently used hosts for demonstrating production of biofuel candidates including alcohol-, fatty acid- and terpenoid-based biofuels. In this review, we summarize the metabolic pathways for synthesis of these biofuels and assess enabling technologies that assist in regulating biofuel synthesis pathways and rapidly assembling novel *E. coli* strains. These advances maintain *E. coli*'s position as a prominent host for developing cell factories for biofuel production.

Addresses
[1] School of Biology and Basic Medical Sciences, Soochow University, Suzhou, People's Republic of China
[2] Department of Chemical and Biological Engineering, University of Wisconsin—Madison, Madison, WI, USA
[3] Microbiology Doctoral Training Program, University of Wisconsin—Madison, Madison, WI, USA
[4] Division of Applied Life Science (BK21 Plus), PMBBRC, Institute of Agriculture and Life Sciences, Gyeongsang National University, Jinju, Republic of Korea

Corresponding authors: Pfleger, Brian F (brian.pfleger@wisc.edu), Kim, Seon-Won (swkim@gnu.ac.kr)

Introduction

The development of biofuel-producing microbes is motivated by a desire to find sustainable alternatives to fossil fuels and to reduce the net amount of greenhouse gases released through the combustion of transportation fuels [1,2]. Microbial metabolism has evolved to comprise pathways for synthesizing a variety of metabolites with fuel-compatible properties including linear, branched and cyclic alcohols, alkanes, alkenes, esters and aromatics. Microbes isolated from environmental samples often possess these metabolic pathways for biofuel synthesis [3,4]; however, they rarely synthesize biofuels in the elevated quantities needed for economically-viable processes. That said, there exist several natural isolates that produce relevant quantities of fuels, for example, efficient fermentation of sugar to ethanol by yeast or to butanol by *Clostridium* species [5], but the probability of identifying a natural isolate capable of producing other advanced biofuels is infinitesimally small. Furthermore, natural hosts rarely possess the complete suite of traits required for commercial biofuel production. Engineering native isolates into robust workhorses often requires overcoming limitations such as low growth rates, limited catabolic capabilities, poor tolerance to substrates, products, and desirable environmental conditions. For this reason, the alternative approach of engineering a model, genetically-tractable host is a common strategy for accessing the repertoire of natural and synthetic biofuel-producing pathways.

With the advent of recombinant DNA technology, harnessing a user-friendly microorganism such as *Escherichia coli* for biofuel production could be an attractive alternative to the natural isolates [6–8]. *E. coli* as a host has several advantages over other industrial microbes for biofuel production (Table 1). The advantages are as follows: (i) both aerobic and anaerobic growth using various carbon sources in defined salt media; (ii) possession of high growth and metabolic rates; (iii) a vast knowledgebase of genetic, metabolic and physiological traits; and (iv) a plethora of genetic tools available for performing metabolic engineering. These benefits encourage metabolic engineers to construct novel pathways to synthesize desired biofuel products in the genetically tractable *E. coli* host. Enormous genomic sequences have been disclosed for new metabolic reactions and are available in public databases, which provides a wealth of gene variants to construct novel and efficient pathways for biofuels production in *E. coli*. In fact, several biofuel candidates with great potential to replace traditional fuels have been investigated for production in *E. coli* over the past two decades. Such an engineering process relies on introduction of one or more genes encoding the enzymes of the pathway, which are orchestrated by tools of systems biology, metabolic engineering, and synthetic biology to effectively convert intracellular metabolites into the target products [9,10]. The pathway enzymes therefore need to be expressed in *E. coli* at a level that provides sufficient pathway activity but prevents wasteful usage of cellular resources needed elsewhere in metabolism. Similar

Table 1

Comparison of *Escherichia coli* with other industrial strains for biofuel synthesis

Strains	Biofuel types			Available genetic tools	Biofuel tolerance
	Higher alcohols	Fatty acids	Terpenoids		
Escherichia coli	• Non-native producer • Engineered for production of many higher alcohols • Titer:143 g/L isopropanol [27], 50.9 g/L isobutanol [28], 30 g/L 1-butanol [29], and so on.	• Non-native producer • Engineered for production of many fatty acid derivatives • Titer: 1.95 g/L fatty alcohols [45•], 0.6 g/L fatty alkanes [46], 1.1 g/L fatty ester [97••], and so on.	• Non-native producer • Engineered for production of many terpenoids via MEP or MVA pathway • Titer: 2.2 g/L isopentenol [61•], 0.53 g/L farnesol [63•], 1.1 g/L bisabolene [91], > 60 g/L isoprene [69], and so on.	Many	• Low tolerance to most biofuels • Many studies on tolerance engineering
Corynebacterium glutamicum	• Non-native producer • Engineered for production of few higher alcohols • Titer: 4.0 g/L isobutanol [113], 2.8 g/L 3-methyl-1-butanol, and 0.37 g/L 2-methyl-1-butanol [114]	• Non-native producer	• Non-native producer • Engineered for valencene production via MEP pathway • Titer: 2.4 mg/L valencene [115]	Not many	• Not clear for biofuel tolerance • Few studies on tolerance engineering
Saccharomyces cerevisiae	• Native ethanol producer • Engineered for isobutanol production • Titer: 1.62 g/L isobutantol [31]	• Non-native producer • Engineered for production of many fatty acid derivatives • Titer: 0.1 g/L fatty alkanes [47], 1.1 g/L hexadecanol [48], and so on.	• Non-native producer • Engineered for production of many terpenoids via MVA pathway • Titer: 0.1 g/L farnesol [64], 1.0 g/L bisabolene [68], >100 g/L farnesene [71], and so on.	Many	• High tolerance to ethanol • Not many studies on tolerance engineering
Clostridium acetobutylicum	• Native butanol producer • Engineered for high butanol production • Titer: 130 g/L butanol [30]	• Non-native producer	• Non-native producer	Few	• High tolerance to butanol • Not many studies on tolerance engineering
Pseudomonas putida	• Non-native producer • Engineered for butanol production • Titer range: 0.12 g/L butanol [21]	• Non-native producer	• Non-native producer • Engineered for geranic acid production via MVA pathway • Titer: 0.19 g/L geranic acid [116]	Not many	• High tolerance to many organic solvents • Few studies on tolerance engineering
Bacillus subtilis	• Non-native producer • Engineered for production of few higher alcohols • Titer: 24 mg/L butanol [21] and 6.1 g/L isobutanol [33]	• Non-native producer	• Native isoprene producer • Engineered for isoprene production via MEP pathway	Not many	• Not clear for biofuel tolerance • No study on tolerance engineering
Yarrowia lipolytica	• Non-native producer	• Native producer • Engineered for production of fatty acid derivatives • Titer: 0.5 g/L decanol [53], 4.98 mg/L pentane [54], and 0.64 g/L hexadecanol [55]	• Non-native producer • Engineered for α-farnesene production via MVA pathway • Titer: 0.26 g/L α-farnesene [117]	Not many	• Not clear for biofuel tolerance • No study on tolerance engineering